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Longitudinal HIV-1 RNA Levels in a Cohort of Homosexual Men

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Summary: HIV-1 RNA levels measured during early chronic infection strongly predict subsequent clinical events. In the short term, HIV-1 is in a steady state, but the stability of viral levels over time is incompletely understood. We used reverse transcriptase polymerase chain reaction (RT-PCR) to examine changes in serum HIV-1 RNA levels in 111 HIV-1–infected homosexual men during the period from 1982 to 1992 and their relation to clinical outcomes. HIV-1 RNA levels increased by a median of 0.08 $\log_{10}$ copies/ml/year ($p = .0001$). HIV-1 RNA levels rose either gradually or abruptly for the majority of subjects; 41% had no increase. Among subjects surviving at least 8 years, HIV-1 RNA was stable during the first 4 years after seroconversion (median, 0.00 $\log_{10}$ copies/ml/year), but rose in years five through eight (median, 0.06 $\log_{10}$ copies/ml/year; $p = .04$). The annual HIV-1 RNA level was more predictive of AIDS (relative hazard [RH], 1.75 per 0.5 log difference; 95% confidence interval [CI], 1.38–2.21; likelihood ratio [LR], 26.2) than the initial level alone (RH, 1.39; 95% CI, 1.10–1.76; LR, 8.5). We conclude that most HIV-1–infected persons lack a long-term viral setpoint and that failure to account for evolution of the viral level can lead to underestimation of the risk of progression. **Key Words:** Longitudinal HIV-1—RNA levels—Homosexual men—AIDS—Epidemiology.

The amount of circulating HIV-1 is high during primary HIV-1 infection (1) but falls to lower levels during long-term infection (2). HIV-1 RNA levels measured during early phases of long-term HIV-1 infection are strong predictors of the AIDS incubation period (3,4), with low levels defining persons with a high probability of long-term AIDS-free survival. Higher HIV-1 RNA levels predict an increased risk of AIDS and death, even after the $CD4^+$ lymphocyte count is considered (2–5).

During long-term infection, HIV-1 RNA levels are in a short-term steady state, the therapeutic perturbation of which allows estimation of the HIV-1 replication rate (6–7). The extent to which HIV-1 RNA levels exist in a long-term steady state (i.e., whether a true "viral setpoint" exists) is unclear. In a cross-sectional study, HIV-1 RNA levels were higher in persons with AIDS than in asymptomatic persons (8), but this difference might simply reflect that the AIDS risk is greater in persons with higher RNA levels. In a previous longitudinal assessment of some subjects included in the present study, HIV-1 RNA levels appeared relatively constant as measured by a semiquantitative assay (2).

The patterns of HIV-1 RNA levels over time have implications for HIV pathogenesis and for the frequency with which HIV-1 RNA levels should be measured in the clinical setting. To examine these patterns and their relation to clinical outcomes, we used a sensitive quantitative HIV-1 RNA assay to determine longitudinal HIV-1 RNA levels in homosexual men enrolled in a long-term cohort study.

METHODS

Subjects

In 1982, 245 homosexual men were enrolled in a National Cancer Institute–sponsored cohort of patients of primary care physicians

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in New York City or Washington, D.C. (9). Subjects were generally observed annually, except for 1983 when the Washington subjects were not seen because of limited funding. Seroconversion was determined as the midpoint between the last negative and the first positive HIV-1 antibody test. For subjects who were seropositive on study entry in 1982, the seroconversion date was estimated to be June 1, 1980 for subjects enrolled in New York City and June 1, 1981 for subjects enrolled in Washington, D.C., on the basis of available information regarding the HIV-1 epidemic in these cities.

HIV-1 RNA measurements from two or more time points were available for 111 of the 131 men who were positive for antibodies to HIV-1 before 1992; 67 of the 111 subjects (60%) were HIV-1–seropositive at study entry (1982); 31 (28%) seroconverted by 1983; and the remaining 13 (12%) seroconverted by 1986. The median age at seroconversion for these 111 subjects was 33 years (interquartile range, 29–38 years). The racial distribution was: white, 89.2%; black, 6.3%; Hispanic, 3.6%; Asian, 0.9%. The median number of HIV-1 RNA measurements taken was five (mean, 5.6 measurements; range, 2–11 measurements). During 1982 to 1992, 64 of the 111 subjects developed documented AIDS, and 65 died.

Laboratory Methods

Blood specimens were collected in offices of primary care physicians and shipped to a central repository for later testing. Serum was separated and frozen within 6 to 24 hours. We considered a serum specimen positive for HIV-1 antibody if it was repeatedly reactive by a commercially licensed HIV-1 enzyme immunoassay (EIA) and positive by HIV-1 Western blot (Cambridge Biotech, Rockville, MD, U.S.A.) or radioimmunoassay (9).

We measured HIV-1 RNA levels from previously unthawed serum specimens using the Amplicor HIV Monitor assay (Roche Molecular Systems, Branchburg, NJ, U.S.A.). With this assay, a 142 base pair sequence in the HIV-1 *gag* gene and an RNA quantitation standard are reverse transcribed and amplified by polymerase chain reaction in a single reaction using rTth DNA polymerase (10). Serial dilutions of the target molecule and the quantitation standard permit measurement of HIV-1 RNA, expressed as $\log_{10}$ HIV-1 RNA copies/ml, over a range of 2.30 to >6.00. HIV-1 RNA measurements <2.30 were assigned a value of 2.00.

The total lymphocyte count was determined by a commercial laboratory that performed an automated white blood cell count and a manual differential count. The percentage of $CD4^+$ lymphocytes was measured by flow cytometry using the whole blood lysate method (11) or methods appropriate for frozen lymphocyte specimens (12). The total $CD4^+$ lymphocyte count was the product of the total lymphocyte count and the percentage of $CD4^+$ lymphocytes.

Data Analysis

Slopes (annualized changes in HIV-1 RNA levels, $CD4^+$ lymphocyte counts, and $CD8^+$ lymphocyte counts from the initial value) were determined by the SAS GLM procedure (13). We used the *t*-test to evaluate whether the mean slope for the population differed from 0.0 $\log_{10}$ HIV-1 RNA copies/ml/year (13), the Wilcoxon rank sum test to compare slopes by length of survival, and the Wilcoxon signed rank test to compare the early slopes to late slopes among 8-year survivors (14). We assessed the relative hazard of AIDS (15) and death over the years following seroconversion in proportional hazards models (16,17). We treated age at seroconversion and the initial HIV-1 RNA level as fixed covariates; the annual $CD4^+$ lymphocyte count, annual HIV-1 RNA level, and the use of antiretroviral therapy were treated as time-dependent covariates (18). Initiation of each nucleoside analogue was assumed to result in no more than 6 months of effective therapy, such that the treatment covariate returned to "untreated" 6 months after initiation of therapy or when treatment with a specific nucleoside analogue was stopped. Models in which the assumed period of effectiveness was longer (1 year or infinite) and a model that excluded therapy produced similar results for the effect of $CD4^+$ count and HIV-1 RNA levels.

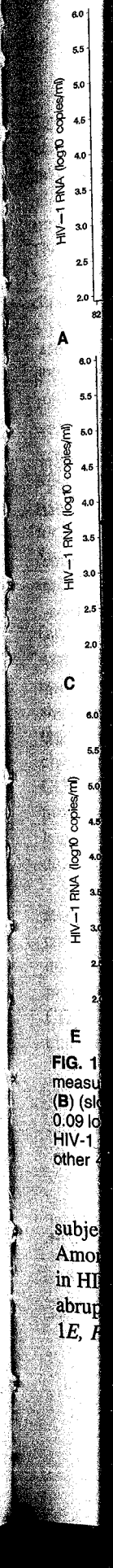
RESULTS

Comparison of Serum and Plasma HIV-1 RNA Levels

Although HIV-1 RNA levels are measured in plasma for clinical purposes, we used serum specimens in this study because plasma was not collected during all years of the study. To evaluate possible differences in HIV-1 RNA levels measured in different types of specimens, we compared levels from plasma (collected in acid citrate dextrose tubes) and serum specimens collected simultaneously in 1996. All samples were measured in duplicate and the mean values for each individual were used to determine population means. Nineteen subjects had detectable virus in the plasma specimen, with a median variation for replicate measurements of 0.15 $\log_{10}$ copies/ml in plasma and 0.19 $\log_{10}$ copies/ml in serum. The mean HIV-1 RNA level was 4.23 $\log_{10}$ copies/ml in plasma and 4.11 $\log_{10}$ copies/ml in serum (correlation coefficient, 0.95; $p = .0001$). Therefore, serum measurements were only slightly lower than those from plasma and the results were very highly correlated.

Individual Trajectories

We evaluated HIV-1 RNA levels in 111 subjects for whom 2 or more measurements were available for the period 1982 to 1992. Examination of this time period had several advantages: subjects who were HIV-1 seropositive at study entry were unlikely to have been infected for a lengthy period; long-term follow-up was possible for many subjects; and therapeutic regimens producing sustained decreases in HIV-1 RNA levels were not yet available. Review of individual trajectories revealed several qualitative patterns (Fig. 1). Some subjects had a series of consistent measures compatible with a steady-state viremia (Fig. 1*A*, *B*), but for other



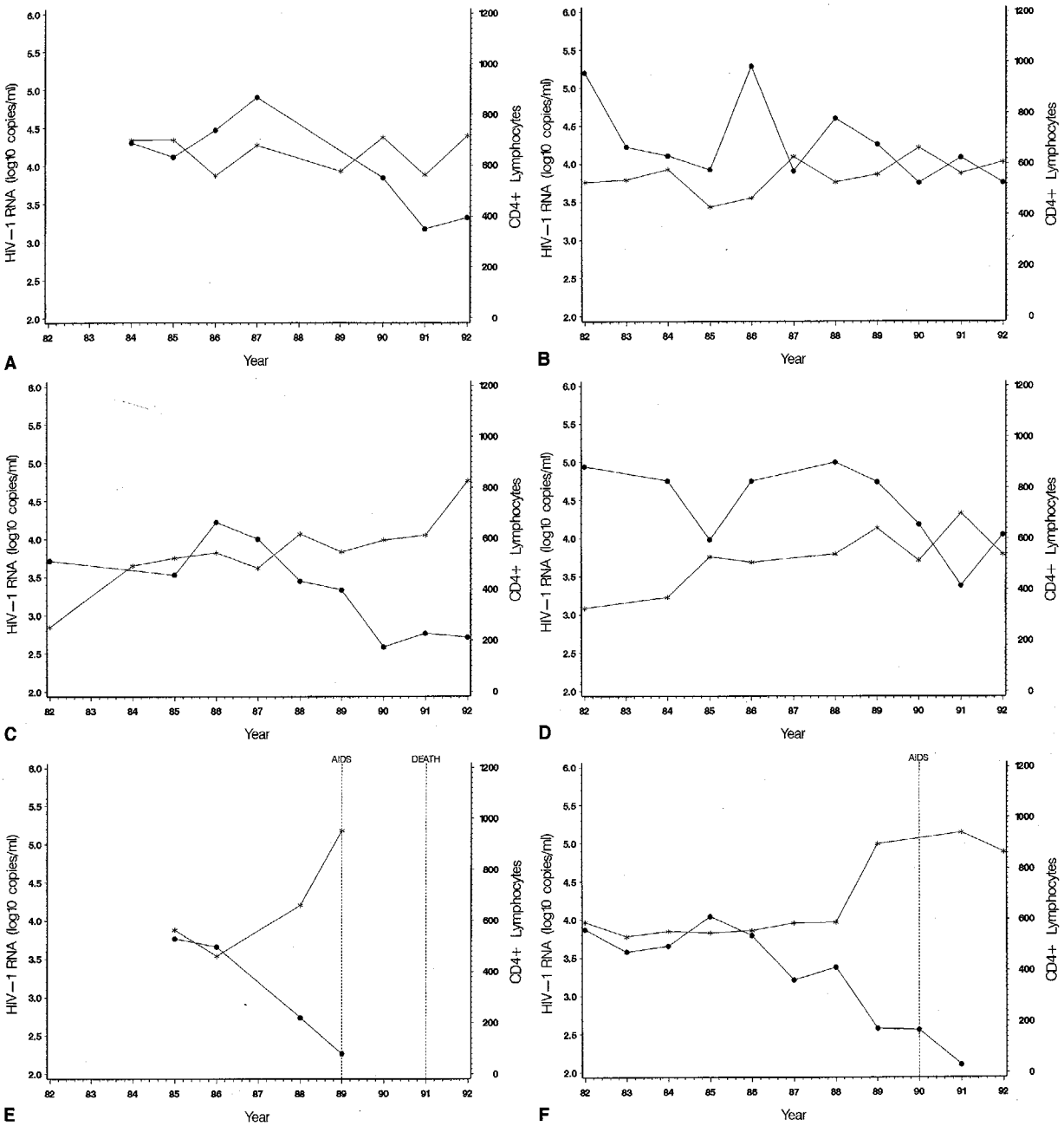


FIG. 1. Longitudinal HIV-1 RNA levels (log_{10} HIV-1 RNA copies/ml) and $CD4^+$ lymphocyte counts in selected subjects, by year of measurement. Observed patterns of HIV-1 RNA levels included: relatively flat (**A**) (slope, −0.01 log_{10} HIV-1 RNA copies/ml/year), and (**B**) (slope, 0.03 log_{10} HIV-1 RNA copies/ml/year); gradually increasing (**C**) (slope, 0.13 log_{10} HIV-1 RNA copies/ml/year), and (**D**) (slope, 0.09 log_{10} HIV-1 RNA copies/ml/year); and abruptly increasing (**E**) (slope, 0.13 log_{10} HIV-1 RNA copies/ml/year) and (**F**) (slope, 0.30 log_{10} HIV-1 RNA copies/ml/year). Subject **A** became seropositive for HIV-1 in 1984, subject **F** became seropositive for HIV-1 in 1985, and the other 4 subjects were seropositive for HIV-1 on study entry in 1982. Asterisks indicate RNA; bullets indicate CD4.

subjects the HIV-1 RNA level increased over time. Among subjects with increasing viral levels, the change in HIV-1 RNA was often gradual (Fig. 1*C, D*), although abrupt increases were observed in some subjects (Fig. 1*E, F*).

Distribution of HIV-1 RNA Slopes

Among 44 subjects with an observed date of seroconversion, the overall median annualized change in HIV-1 RNA from the initial value (HIV-1 RNA slope) was 0.06

$\log_{10}$ HIV-1 RNA copies/ml/year (mean, 0.15 $\log_{10}$ copies/ml/year; $p = .001$). With the inclusion of 67 subjects with imputed dates of seroconversion (total subjects, 111) the median was 0.08 $\log_{10}$ HIV-1 RNA copies/ml/year (Table 1); similar results were obtained when we eliminated the first HIV-1 RNA measurement (to control for the possibility that the initial level was obtained close to the time of primary infection when viral levels tend to be higher) for the seroconverters or for all 111 subjects. In subsequent analysis, we present all measurements from all 111 subjects except as indicated. Examining the distribution of slopes (Fig. 2), 20 (18%) subjects had a slope less than -0.1 $\log_{10}$ copies/ml/year, 26 (23%) had a slope of 0.0, 33 (30%) had a slope of 0.1, 14 (13%) had a slope of 0.2 and 18 (16%) had a slope of ≥ 0.3. Thus, HIV-1 RNA levels rose for most subjects, although a sizable minority either had no increase or had a decrease over time. No correlation existed between the initial HIV-1 RNA and HIV-1 RNA slope among all subjects or among subjects with greater slopes.

We also examined slopes over shorter intervals, limiting the analysis to persons surviving to the end of selected time periods (Table 1). Among 98 subjects who survived at least 4 years postseroconversion and had two or more measurements during that period, the median 4-year slope was 0.06 copies/year (mean, 0.05 copies/year). The median 6-year slope ($n = 87$) was also 0.06 copies/year (mean, 0.06 copies/year; $p = .01$). Therefore, during these relatively early periods, the HIV-1 RNA level was increasing for most subjects. The median slope for 8 years, as well as for 10 years, was 0.05 $\log_{10}$ copies/ml/year. The slopes for each interval differed significantly from zero, with the exception of the 4-year period.

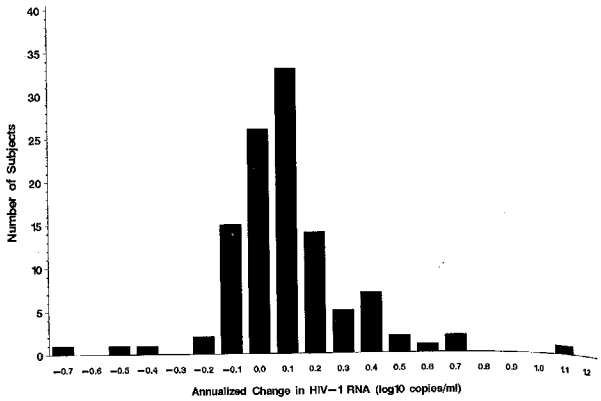


FIG. 2. Distribution of annualized change in $\log_{10}$ HIV-1 RNA level (slope) in 111 HIV-1–infected homosexual men. Slopes are presented to the nearest 0.1 $\log_{10}$ HIV-1 RNA copies/ml/year.

To examine whether slopes were constant over time for longer-term survivors, we compared early and late period values for 8-year survivors with at least 2 measurements during each period (Table 1). The median was 0.00 $\log_{10}$ copies/ml/year during the first 4 years after seroconversion and 0.06 $\log_{10}$ copies/ml/year during years five through eight ($p = .04$). Therefore, for longer-term survivors, slopes tended to be flat for several years before beginning to rise.

TABLE 1. *HIV-1 RNA slope (annualized change in $\log_{10}$ HIV-1 RNA copies/ml) during selected time periods after seroconversion in a cohort of HIV-1-infected homosexual men, 1982–1992*

Years	No. of subjects	HIV-1 RNA slope					
		25th %	50th %	75th %	Mean	p Value[a]	p Value[b]
All	111	−0.01	0.08	0.17	0.10	.0001	
1–4	98	−0.14	0.06	0.22	0.05	.2	
1–6	87	−0.07	0.06	0.17	0.06	.01	
1–8	62	−0.04	0.05	0.12	0.06	.004	
1–10	43	−0.04	0.05	0.10	0.04	.02	
8-Year survivors							
1–4	53	−0.23	0.00	0.14	−0.05	.2	.04
5–8	53	−0.05	0.06	0.23	0.08	.08	
Slope in Years 1–4, by years of survival							
≥6	82	−0.17	0.05	0.20	0.03	.5	
4–6	16	−0.06	0.14	0.32	0.16	.1	.2
Slope in Years 1–6, by years of survival							
≥8	60	−0.09	0.02	0.15	0.03	.3	
6–8	27	0.00	0.13	0.31	0.13	.01	.03

Date of HIV-1 seroconversion was based on last negative and first positive HIV-1 antibody tests for seroconverters and imputed for subjects whose serum had antibodies to HIV-1 at study entry in 1982.

[a] Compared with mean slope of 0.0 $\log_{10}$ HIV-1 RNA copies/ml/year.

[b] Compares slopes by period of observation or by length of survival of the subjects.

Longitudinal HIV-1 RNA Levels and Clinical Outcomes

As one measure of the relation between HIV-1 RNA slope and clinical outcome, we compared slopes of subjects with different lengths of survival (Table 1). The median 4-year slope was 0.05 $\log_{10}$ HIV-1 RNA copies/ml/year among 6-year survivors and 0.14 copies/year among subjects who survived 4, but not 6 years ($p = .2$). The median 6-year slope was 0.02 copies/year among 8-year survivors, compared with 0.13 $\log_{10}$ HIV-1 RNA copies/year among subjects who survived 6 years but not 8 years ($p = .03$).

To examine further the association between HIV-1 RNA increases and clinical outcomes, we determined the 1982 to 1986 HIV-1 RNA slope for 43 subjects who were infected before study entry (1982), but remained free of AIDS through 1986 (Table 2). The initial HIV-1 RNA level was a stronger predictor of post-1986 AIDS risk than the slope, but the risk of AIDS increased 37% for each 0.10 $\log_{10}$ HIV-1 RNA copies/year increase. Similarly, the post-1986 mortality risk increased 25% for each 0.10 $\log_{10}$ copies/year increase.

The rate of $CD4^+$ lymphocyte depletion in the cohort (mean, 64 cells/mm^3/year; median, 53 cells/mm^3/year) was correlated with the RNA slope with a correlation coefficient (R) of −0.26 ($p = .006$). This correlation was slightly stronger when the square root transformation of the $CD4^+$ lymphocyte count, which tends to linearize changes, was used ($R = -0.31$, $p = .001$). The rate of $CD8^+$ lymphocyte depletion (mean, 17 cells/mm^3/year; median, 3 cells/mm^3/year) was not significantly correlated with HIV-1 RNA slope ($R = -0.13$; $p = .2$) and did not differ with the square root transformation.

Because increasing HIV-1 RNA levels were associated with a poorer prognosis, we created time-dependent covariate models to compare the predictive value of annual HIV-1 RNA measurements to that of the initial measurement alone (Table 3). Model A included annual $CD4^+$ lymphocyte counts only, whereas model B included the initial HIV-1 RNA level and annual $CD4^+$ lymphocyte counts. Consistent with previous studies, model B predicted AIDS risk much better than model A ($p = .005$, likelihood ratio [LR] test). However, model C (annual measurements of both $CD4^+$ lymphocytes and HIV-1 RNA levels) was more predictive of AIDS risk than model B ($p = .0001$, LR test) and produced a higher HIV-1 RNA-specific relative hazard. Time-dependent covariate models of the risk of death produced similar results (data not presented).

The substantive difference between models B and C requires consideration of increasing HIV-1 RNA levels over time. This difference may be appreciated by comparing the AIDS risk of a hypothetical person with an HIV-1 RNA level of 4.0 $\log_{10}$ copies/ml at baseline and a 5-year slope of 0.10 $\log_{10}$ HIV-1 RNA copies/year to a person with 3.0 $\log_{10}$ copies/ml at baseline and a 5-year slope of 0.00 $\log_{10}$ HIV-1 RNA copies/year. Five years after the initial measurement, the first person would have a 2.0-fold greater risk of developing AIDS than the second person under model B, but a 5.2-fold greater risk under model C. Failure to account for evolution of the viral level would lead to underestimation of the individual risk of progression.

DISCUSSION

In this cohort of HIV-1–infected homosexual men, serum HIV-1 RNA measurements in specimens collected between 1982 and 1992 displayed a range of patterns. Although many subjects had little or no increase in the HIV-1 RNA level, the overall median increase was 0.08 $\log_{10}$ copies/year. For many subjects, the HIV-1 RNA level increased during the first 4 years of infection, but for longer-term survivors, increases tended to occur later. Although in some cases the HIV-1 RNA level increased abruptly, for many subjects the increase was gradual. Our data indicate, therefore, that a long-term steady-state viremia does not exist for most HIV-1–infected individuals.

During the short-term steady state, HIV-1 production

TABLE 2. *Unadjusted and adjusted relative hazards (RH) and 95% confidence intervals (95% CI) of developing AIDS or dying after 1986, by initial HIV-1 RNA measurement and HIV-1 RNA slope during 1982–1986*

	AIDS ($n = 43$)		Death ($n = 54$)	
	RH (95% CI)	Adjusted RH (95% CI)[a]	RH (95% CI)	Adjusted RH (95% CI)[a]
Initial HIV-1 RNA (per 0.5 log)	1.94 (1.43–2.67)	2.32 (1.63–3.30)	1.63 (1.24–2.15)	1.79 (1.36–2.34)
HIV-1 RNA slope (per 0.1 log/year)	1.11 (0.91–1.35)	1.37 (1.08–1.73)	1.12 (0.94–1.33)	1.25 (1.02–1.52)

Subjects were HIV-1–infected before entry into the study in 1982.
[a] Based on a multivariate model that included initial HIV-1 RNA ($\log_{10}$ copies/ml), HIV-1 RNA slope (annualized change in HIV-1 RNA), and age as continuous variables.
CI, confidence interval; RH, relative hazard.

TABLE 3. *Time-dependent analysis of adjusted relative hazard (RH) of AIDS, 1982–1992*

	RH (95% CI)	Likelihood ratio[a]	*p* Value
Model B			
HIV-1 RNA (initial) (per 0.5 log difference)	1.39 (1.10–1.76)	8.5	.005
$CD4^+$ count (annual) (per 50-cell difference)	1.25 (1.16–1.34)		
Antiretroviral therapy[b]	0.62 (0.21–1.78)		
Model C			
HIV-1 RNA (annual) (per 0.5 log difference)	1.75 (1.38–2.21)	26.2	.0001
$CD4^+$ count (annual) (per 50-cell difference)	1.17 (1.09–1.26)		
Antiretroviral therapy[b]	0.55 (0.19–1.57)		

[a] Likelihood ratio and *p* value compare model B or model C to a model A (data not presented) that includes annual $CD4^+$ lymphocyte count and antiretroviral therapy only. All 3 models include age at seroconversion.

[b] Forty-six subjects received nucleoside analogue monotherapy during the period of analysis.

CI, confidence interval; RH, relative hazard.

and clearance are balanced by mechanisms that are incompletely understood (19). Our observations demonstrate that this quasi steady state changes over time, presumably because viral production increases or viral clearance decreases. Although $CD4^+$ lymphocytes are depleted during the course of infection, HIV-1 production might increase if the number of activated $CD4^+$ lymphocytes, the source of most replication, increase or if the number of virions produced per infected $CD4^+$ lymphocyte increase. Viral production by other HIV-1–infected cell populations (e.g., macrophages) also might increase with time, although if HIV-1 replication in these cells accounts for <1% of the HIV-1 found in peripheral blood (20), this source alone could not explain the increases in HIV-1 RNA that we observed. Alternatively, our observations could reflect the immune system's declining ability to clear virus. Recent studies, however, found no relation between a person's CD4 count and their rate of viral clearance (6,20). Clearly, these basic questions of HIV-1 pathogenesis deserve further study.

We and others have previously shown that a single HIV-1 RNA measurement from early in the course of infection strongly predicts the risk of AIDS and death (2–4). Our present study demonstrates that annually measured HIV-1 RNA levels predict AIDS risk better than an initial level alone. Estimates of absolute AIDS risk based on a single determination of the HIV-1 RNA level and of the $CD4^+$ lymphocyte count have been proposed (21). Our findings do not invalidate these estimates, but rather indicate that increasing HIV-1 RNA levels, as well as decreasing $CD4^+$ lymphocyte counts, may cause an individual's prognosis to worsen over time. Patients who choose to delay receiving antiretroviral therapies should have regular prognostic assessments based on current HIV-1 RNA and $CD4^+$ lymphocyte measurements.

An earlier analysis of HIV-1 RNA in this cohort revealed less evidence that levels increase with time (2). The difference between the results of that study and the present one likely reflects better sensitivity and precision of the assay used in the current study. As the Monitor assay measures HIV-1 RNA on a continuous scale with an analytic sensitivity of 2.30 $\log_{10}$ HIV-1 RNA copies/ml, almost all measurements were now quantifiable.

Some potential limitations of our study should be considered. We measured HIV-1 RNA levels in serum specimens rather than plasma because the latter was not collected at each study visit. Our comparison data indicated, however, that serum levels are only slightly lower than plasma levels and that the results are highly correlated. We included subjects with imputed seroconversion dates in the analysis to increase its statistical power and the length of follow-up. The rate of HIV-1 RNA increase was similar among observed seroconverters and subjects with imputed dates. Therefore, neither the use of serum nor the inclusion of subjects with imputed dates of seroconversion is likely to have qualitatively altered our results.

Although our period of analysis ended before highly effective antiretroviral regimens became available, 52 (47%) subjects received monotherapy with a nucleoside analogue during the latter part of this analysis. Because such therapy temporarily decreases HIV-1 RNA levels by about 0.5 $\log_{10}$ copies/ml (22), we likely underestimated the true HIV-1 RNA slope for some subjects and the difference in HIV-1 RNA slope over time in longer-term survivors. Use of antiretroviral therapy may explain, at least in part, why some subjects had negative HIV-1 RNA slopes.

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